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ROLE OF OXIDATIVE STRESS AND ANTIOXIDANTS IN AETIOPATHOGENESIS AND MANAGEMENT OF ORAL SUBMUCOUS FIBROSIS

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ABSTRACT

Lipid peroxidation product, malonaldehyde (MDA) and antioxidants were estimated in plasma and erythrocytes of 34 cases of oral submucous fibrosis (OSMF) of different grades with equal number of healthy controls to evaluate the association of reactive oxygen species (ROS) and OSMF. While plasma MDA was found to be significantly higher in patients (3.3. $\pm$ 0.4 nmole/ml, $P < 0.001$) as compared to controls (2.4 $\pm$ 0.5 nmole/ml), plasma beta carotene and vitamin E levels were found to be decreased significantly in patients (81.7 $\pm$ 14.3 µg/100 ml, $P<0.001$; 9.3 $\pm$ 0.9 mg/L, $P<0.01$ respectively) with respect to healthy controls (110 $\pm$ 20.8 µg/100ml and 10.1 $\pm$ 1.2 mg/L). The decrease in beta-carotene and vitamin E was found to be more significant in OSMF grade II and III than in grade I. After 6 weeks of oral administration of beta-carotene and vitamin E, patients showed increase in plasma level of these two antioxidants along with decrease in MDA level associated with clinical improvement.

KEY WORDS

Beta-carotene, MDA, OSMF, ROS, Vitamin E

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic insidious and progressive disease involving oral mucosa. Recent survey shows that there is an increase in prevalence of OSMF in different states of India (1). The condition has been shown to be precancerous (2) and carries a high relative risk for malignant conversion even after the control of tobacco use (3), which is known to play a major role in the development of the disease (4). There is no report suggesting spontaneous regression and there is no effective or widely accepted treatment making the situation worse (5). The prevalence of OSMF in this area is second highest in the country (6).

Epidemiological studies have shown that the process of carcinogenesis occurs by generation of Reactive Oxygen Species (ROS) (7), which act by initiating lipid peroxidation (LPO)(8). Prevention against LPO mediated damage is done by non-enzymatic antioxidants, especially Beta-carotene and Vitamin E and enzymatic antioxidant like superoxide dismutase (SOD)(9). It has also been reported that leukoplakia, an oral premalignant condition can be successfully treated by antioxidant supplementation (10).

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OSMF, being a premalignant condition and associated with carcinogens like tobacco was thought to have some relation with ROS. Hence the present study was undertaken to assess blood levels of lipid peroxidation product and antioxidant defense system in OSMF cases. The study was also aimed to reevaluate the patients clinically and biochemically after supplementing the patients with antioxidant therapy.

MATERIALS AND METHODS

A total of 34 cases of OSMF attending out patient department of Otolaryngology of this Institute along with equal number of age and sex matched healthy normal individuals were included in this study. The OSMF cases were divided into Grade I (n = 13) Grade II (n = 17) and Grade III (n = 4) using the criteria of Bhatt and Dholakia (11).

All patients were subjected to a thorough history regarding dietary habits and addiction. They were clinically examined by measuring mouth bite (the maximum distance between upper and lower central incisor teeth) and tongue protrusion as described by Kumar and Srivastava (12).

From each individual about 5 ml of blood was collected using EDTA as anticoagulant. Plasma was separated and preserved at -20°C. Buffy coat was removed and erythrocytes were washed 3 times in cold saline (9 g/L) and packed erythrocytes were stored in 500 µl aliquots at -20°C . Plasma was used to estimate MDA, the major lipid peroxidation product by

thiobarbituric acid (TBA) reaction using the method of Kei Satoh (13). Beta-carotene and vitamin E were also estimated in plasma by the method of Bieri *et al* (14). Erythrocytic SOD activity was estimated from packed RBC by pyrogallol autooxidation method of Marklund and Marklund (15).

The patients were advised to stop chewing betel nut, tobacco, pan masala, to quit smoking and to maintain proper oral hygiene. Pharmacological preparation containing only beta-carotene and Vitamin E is not available. Hence they were treated with "antoxid" tablets manufactured by American Remedies soft caps private limited, 1 tablet thrice daily for 6 wks. Each tablet contains beta-carotene 50 mg, Vitamin A palmitate 2500 IU, Vitamin E acetate 10 IU along with Vitamin C, zinc manganese and copper.

The patients were reevaluated in terms of amelioration of symptoms, specifically burning sensation in the mouth, improvement in mouth bite and tongue protrusion, change in colour and consistency of oral mucosa and regression in the fibrous band.

The results were expressed as Mean ± SD values. The means of the controls and patients were compared using student's 't' test.

RESULTS AND DISCUSSION

The history of the patients revealed that a significant portion of OSMF cases (67.8%) chewed tobacco with or without pan and areca nut. 15.5% of the patients were smokers and 79.3% were non-vegetarian. The age of patients varied from 19 yrs. to 47 yrs, mean age being 30.7 yrs.

Plasma MDA level was found to be increased in all grades of OSMF cases (mean = 3.3 ± 0.4 nmole/ml) compared to healthy controls (mean = 2.4 ± 0.5 nmole/ml) and the increase was statistically significant ($P < 0.001$) (Table 1). It is established that the lipid peroxidation increases with severity of the disease reflecting the extent of tissue injury (16). Our results also show that the mean MDA level is more in OSMF grade II than in grade I. However, it did not vary much between grade II and III (Table 2).

Table 1

Levels of malonaldehyde (MDA) and antioxidants in oral submucous fibrosis cases and healthy controls.

Parameters	Healthy controls (n = 34)	OSMF cases (n = 34)	P value
MDA (n mole/ml)	2.4 + 0.5	3.3 + 0.4	< 0.001
Beta Carotene (ug/ 100ml)	110 + 20.8	81.7 + 14.3	< 0.001
Vitamin E (mg / L)	10.1 + 1.2	9.3 + 0.9	< 0.01
E-SOD (u/g Hb)	787.2 + 46.2	807.8 + 66.2	Not significant

Table 2

Levels of malonaldehyde (MDA) and antioxidants in different grades of oral submucous fibrosis.

Parameters	Controls (n = 34)	OSMF Gr : I (n = 12)	OSMF Gr : II (n = 17)	OSMF Gr : III (n = 4)
MDA (n mole/ml)	2.4 ± 0.5	3.1 ± 0.8*	3.5 ± 0.8*	3.3 ± 0.1*
Beta Carotene (mg/ 100ml)	110 ± 20.8	93 ± 24.2#	79.3 ± 13.7*	72.8 ± 5.1*
Vitamin E (mg / L)	10.1 ± 1.2	10.0 ± 1.0	8.8 ± 0.7*	8.5 ± 1.2*
E-SOD (U/g Hb)	787.2 ± 46.2	790.9 ± 55.5	820.1 ± 45.7	812.4 ± 27.7

* $P < 0.001$ as compared to healthy controls

\# $P < 0.02$ as compared to healthy controls

Non-enzymatic antioxidant defense status of the body was assessed by plasma beta-carotene and Vitamin E level. Beta-carotene is known to act by trapping and quenching ROS, while Vitamin E is known to be the most potent fat-soluble chain breaking antioxidant (17). In our study beta carotene level was found to be decreased in all grades of OSMF cases (mean = 81.7 ± 14.3 mg/100ml) compared to healthy control (mean = 110 ± 20.8 mg/100ml), the decrease being more in grade II and grade III cases (Table - 2).

Plasma Vitamin E level was found to be decreased in grade II and III OSMF cases but not in grade I cases. (Table 2) However, mean Vitamen E level was found to be decreased (9.3 ± 0.3 mg/L) as compared to healthy controls (mean = 10.1 ± 1.2 mg/L) (Table 1). Enzymatic antioxidant defense was assessed by SOD activity, which did not show any significant change in any stage of the disease.

Borle *et al* (18) reported that Vitamin A, 50,000 IU chewable tablets, if given once daily could cause symptomatic improvement. Trismus (Tonic spasm of masticatory muscles) did not improve with this treatment. Maher *et al* (19) evaluated the role of multiple micronutrients consisting of retinol, vitamin E, vitamin D, vitamin B complex and some minerals in the management of OSMF and reported clinical improvement. But no conclusive statement was available regarding aetiological correlation of any specific micronutrient and hence their usefulness of administration in management of OSMF. Roth *et al* (20) have shown that turmeric has a beneficial role in treatment of OSMF. Curcuminoids isolated from turmeric, has been found to have effective antioxidant, DNA-protectant and antimutagen actions (20). Arecoline, the major alkaloid of areca nut was shown to be cytotoxic to human buccal fibroblasts in a dose-dependent manner by reducing glutathione S transferase activity. The addition of extracellular nicotine acted synergistically on the arecoline-induced cytotoxicity as was evidenced by increase in lipid peroxidation (21) An *in vitro* study done by Chang *et al* (22) concluded that intracellular thiol depletion could be the mechanism for nicotine toxicity. Addition of 2 oxothiazolidine 4 carboxylic acid, an antioxidant and precursor of cysteine, that metabolically promotes glutathione synthesis was reported to act as a protective agent on the nicotine induced cytotoxicity.

In our study, follow up of 6 OSMF cases after 6 wks treatment showed some improvements and amelioration of the symptoms. There was increase in mouth opening and tongue protrusion measurement. Further in this study the decrease in mean MDA level and the increase in levels of beta-carotene were found to be statistically significant. (Table 3), which show that the assay of levels of beta-carotene, vitamin E and MDA can be useful to monitor the oxidative stress in OSMF cases for better management. Thus the present study shows possibility of ROS playing a part in aetiopathogenesis of the disease, OSMF and administration of nutrient antioxidants may have protecting effect with clinical improvement. Long term follow up study of the patients to fix the dose and duration of therapy is necessary to validate the use of such therapy in OSMF cases and prevent malignant conversion.

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Table 3

Clinical and Biochemical changes in oral submucous fibrosis cases following antioxidant therapy.

Variates	Before treatment (n = 6)	After treatment (n = 6)
Mouth opening (mm)	16 ± 0.7	24 ± 0.3
Tongue protrusion (mm)	19 ± 0.3	24 ± 0.3
MDA (n mole/ml)	3.0 ± 0.5	2.3 ± 0.3**
Beta carotene (ug/100 ml)	86.0 ± 9.8	120.0 ± 10.2*
Vitamin E (mg/L)	8.4 ± 1.1	9.6 ± 0.9

** $P < 0.01$ as compared to patients before treatment.

* $P < 0.001$ as compared to patients before treatment.

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